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ELECTROLYTE AND GAS EXCHANGE DURING THE MOULTING CYCLE OF A FRESHWATER CRAYFISH

BY MICHELE G. WHEATLY

Department of Zoology, University of Florida, Gainesville, FL 32611, USA

AND LORI A. IGNASZEWSKI

Department of Medical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32611, USA

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Summary

Whole-animal net electrolyte fluxes (Ca^{2+}, apparent H^+, titratable acidic equivalents, ammonia, Na^+, Cl^-, K^+, Mg^{2+}, phosphate and sulphate) and respiratory gas exchange were monitored throughout the moulting cycle in juvenile freshwater crayfish *Procambarus clarkii* (Girard) at 21°C. Intermoult crayfish were essentially in ion balance. As crayfish approached ecdysis (−3 days, where t=0 is the day when the cuticle is shed), there was a net efflux of Ca^{2+} ($-1000\,\mu mol\,kg^{-1}\,h^{-1}$) correlated with a corresponding uptake of acidic equivalents (or base output) of $+2000\,\mu mol\,kg^{-1}\,h^{-1}$. Following ecdysis, both fluxes switched vector; uptake of Ca^{2+} ($+2000\,\mu mol\,kg^{-1}\,h^{-1}$) and basic equivalents ($+4000\,\mu mol\,kg^{-1}\,h^{-1}$) were completed within 6 days. The moulting cycle also affected fluxes of electrolytes other than those involved in $CaCO_3$ resorption and deposition. Crayfish remained in Na^+ and Cl^- balance from intermoult up to ecdysis. Following ecdysis, both were taken up actively at rates of around $+500\,\mu mol\,kg^{-1}\,h^{-1}$ for 3 days, presumably restoring the haemodilution that would have resulted from water loading. A premoult efflux of K^+ was partially offset by postmoult uptake. Meanwhile, crayfish experienced increased efflux of phosphate following ecdysis, probably because of increased integumentary permeability. Rates of O_2 uptake ($\dot{M}_{O_2}$) and CO_2 excretion ($\dot{M}_{CO_2}$) increased to peak values (double intermoult rates) immediately prior to ecdysis. While $\dot{M}_{O_2}$ recovered during postmoult, $\dot{M}_{CO_2}$ dropped precipitously, significantly reducing the gas exchange ratio. Since the $\dot{M}_{CO_2}$ deficit agreed well with the postmoult basic equivalent uptake, the latter is probably attributable to HCO_3^- uptake for calcification.

Introduction

The predominant cuticular mineral in crustaceans is $CaCO_3$. Studies of ionoregulation during the crustacean moulting cycle have focused on calcium

Key words: crayfish, moulting, calcium, acid–base balance.

(reviewed by Greenaway, 1985) and only recently on the dynamics of carbonate (Roer and Dillaman, 1984). Intermoult crustaceans are generally in Ca^{2+} balance. During the premoult period, exoskeletal Ca^{2+} is reabsorbed from the old cuticle and either lost to the environment or partially conserved, which happens in species that live in Ca^{2+}-limited environments such as fresh water or on land. At ecdysis the old exoskeleton is shed and Ca^{2+} remaining in it lost. Calcification of the new exoskeleton begins shortly after ecdysis using external and/or stored sources of Ca^{2+}. The mechanism of Ca^{2+} uptake is located in the gills and is probably analogous to that of fish, where a Ca^{2+}-ATPase has been implicated (see model of Flik *et al.* 1985). Studies have suggested that both metabolic and environmental CO_2 (probably in the form of bicarbonate) are the source of carbonate required for $CaCO_3$ formation (Roer and Dillaman, 1984; Cameron and Wood, 1985). In the process, protons are produced *via* the reaction:

$$Ca^{2+} + HCO_3^- = CaCO_3 + H^+$$

and these protons require a mechanism for outward transport (Cameron, 1985).

While Ca^{2+}, HCO_3^- and H^+ fluxes are important in mineral reabsorption/deposition, moulting will also indirectly affect the regulation of other ions, more so in freshwater than in marine species. The increase in size during ecdysis is accomplished by uptake of external water. For freshwater species such as the crayfish, which normally hyperregulate extracellular osmolality and ion concentrations, this will cause haemolymph dilution. Thus during postmoult, in addition to accumulating $CaCO_3$, circulating levels of major electrolytes such as Na^+ and Cl^- must be restored. In fact, there is preliminary evidence of coupled Na^+/Ca^{2+} exchange across mammalian basolateral membranes (Taylor and Windhager, 1979) and in an isolated cuticular crab hypodermis (Roer, 1980); however, it has not yet been demonstrated in branchial Ca^{2+} transport in fish (Flik *et al.* 1985). Another feature of moulting that could potentially affect ion balance is the increase in cuticular permeability around the time of ecdysis, which has been demonstrated for respiratory gases (Mangum *et al.* 1985) and would be expected to increase diffusional ion efflux.

Calcium balance at various stages in the moulting cycle has been documented in the freshwater crayfish by McWhinnie (1962) and Greenaway (1974*a*,*b*,*c*). Greenaway (1974*c*) alluded to the necessity for Na^+ and Cl^- uptake during postmoult. He also hypothesized that the HCO_3^- for calcification originated both externally and from metabolic CO_2, since removal of external HCO_3^- reduced but did not eliminate Ca^{2+} uptake. This was confirmed by Cameron and Wood (1985), who demonstrated apparent H^+ excretion in postmoult blue crabs which matched a net CO_2 deficit (negative CO_2 excretion). CO_2 excretion has not been measured during the moulting cycle in the freshwater crayfish, although Scudamore (1947) measured variations in the rate of O_2 uptake.

The purpose of the present study was to correlate net Ca^{2+} fluxes throughout the moulting cycle of the freshwater crayfish with fluxes of acidic/basic equivalents, major haemolymph electrolytes and respiratory gases.

Materials and methods

Animals and protocols

Measurements were made on 32 juvenile crayfish (mean mass±S.E.M., 1.58±0.18 g) *Procambarus clarkii*, which were obtained from Louisiana State University Agricultural Centre, Baton Rouge. In Gainesville they were kept 10 to a 30-l aquarium under a 12 h light: 12 h dark cycle at 21 °C. The water was recycled through a bottom filter and was replaced every 3 days with dechlorinated thermoequilibrated tapwater. Local tapwater had the following ionic composition (in mmol l^{-1}): Na^+, 0.55; K^+, 0.04; Ca^{2+}, 0.58; Mg^{2+}, 0.43; Cl^-, 0.73; phosphate, 0.003; titration alkalinity 1.80; pH 7.8.

Under these laboratory conditions, this size class moulted naturally approximately every 21 days. Whole-body net electrolyte fluxes and gas exchange rates were measured as a function of time before (t=−7, −3 or −1 days; premoult) or after (t=1–6 days; postmoult) ecdysis (t=0 days) and compared with intermoult values (more than 10 days prior to ecdysis). Premoult was determined by viewing the degree of development of new setae on the uropods and telson (Stevenson, 1985). [For comparison with Drach's (1939) moulting stages, t=−3 and −1 days in this study correspond to stage D1–3, t=+1 day to stage A, t=+2 to +4 days to stage B and t=+6 days to stage C; however, these stages tend to be somewhat arbitrary.]

To determine net whole-body electrolyte fluxes, appropriately staged crayfish were placed individually in experimental chambers containing a fixed volume of tapwater. Chambers were individually aerated and visually shielded. Crayfish remained in the flux water for a total of 24 h and electrolyte concentrations were determined in water samples removed at the start and end of this period. Fluxes were initially determined on 12 crayfish whose mean mass was 1.66±0.18 g. (Mass increased by 7.2±2.1 % after ecdysis in this size class.)

Intermoult flux rates of all ions dictated a flux volume of 50 ml. However, it became apparent after these initial experiments that postmoult Ca^{2+} uptake was potentially limited in such a small volume, since ambient concentration fell below the saturation level for the uptake mechanism (Greenaway, 1974*c*). Rather than reduce the length of the flux period, thereby increasing handling stress, we increased flux volume to 500 ml in a second group of postmoult crayfish (mean mass 1.74±0.20 g, N=8) and remeasured Ca^{2+} and acidic equivalent fluxes. In a third series we remeasured postmoult fluxes of other ions (Na^+, Cl^-, etc.) in a volume of 150 ml to determine whether these were affected by external Ca^{2+} or flux volume. Since no effect could be demonstrated we have reported the original postmoult data for these ions.

Whole-animal rates of O_2 uptake ($\dot{M}_{O_2}$) and CO_2 excretion ($\dot{M}_{CO_2}$) were determined by continuous-flow respirometry as outlined in Wheatly (1989*a*) on 12 crayfish of mean mass 1.71±0.14 g. (In this group mass increased by 9.5±1.4 % after ecdysis.) Respirometers (volume 10 ml) were manufactured from 20 ml disposable syringes. These were fed with air-equilibrated water from a Gilson

minipulse2 pump at a constant flow rate of $1\,ml\,min^{-1}$. Incurrent (inc) and excurrent (exc) flows were sampled for O_2 tension (P_{O_2}) and total CO_2 content (C_{CO_2}). Crayfish were placed in the experimental apparatus at least 6 h before sampling to allow the animal to settle and the respirometer to equilibrate. Five successive pairs of incurrent and excurrent samples were then removed over a period of 3 h to compute a mean value. All respirometers were tested for diffusive gas entry and adequate mixing.

Analytical techniques

Water total ion concentrations of Na^+, K^+, Ca^{2+} and Mg^{2+} were measured using an atomic absorption spectrophotometer (Perkin Elmer model 5000) and chloride ion concentration by coulometric titration (Radiometer CMT 10). Sulphate concentration was determined by the turbidometric method of Jackson and McCandless (1978) and phosphate concentration by the phosphomolybdate method of Atkinson *et al.* (1973). Titration alkalinity was determined by titrating an air-equilibrated 10 ml sample to pH 4 with $0.02\,mol\,l^{-1}$ HCl (McDonald and Wood, 1981) and ammonia ($NH_3+NH_4^+$) using the phenolhypochlorite method (Solorzáno, 1969).

Water P_{O_2} was measured using a thermoequilibrated O_2 electrode (IL 20984) connected to an IL 213 blood gas analyser. Flow rate through the respirometer was adjusted so that the drop in P_{O_2} never exceeded 4 kPa. C_{CO_2} was measured using the Capni-Con (Cameron Instruments Co.) adapted for use with water samples, as outlined by the manufacturer and Cameron and Wood (1985).

Calculations

Net flux rate of electrolyte X was calculated in $\mu mol\,kg^{-1}\,h^{-1}$ as:

$$J^X_{net} = \frac{([X]_i - [X]_f)V}{tW}, \tag{1}$$

where i and f refer to initial and final water concentrations ($\mu mol\,ml^{-1}$), V is flux volume (ml), t is elapsed time (h) and W is mass (kg). A negative value indicates net loss and *vice versa*. By reversing the i and f terms, the net titratable acidity (TA) flux could be calculated from the titratable alkalinities. The net flux of acidic equivalents ($J^{H^+}_{net}$, also termed 'apparent H^+ flux') was calculated as the sum of the titratable acidity and ammonia components (McDonald and Wood, 1981). This method does not distinguish between excretion of acidic equivalents and uptake of basic equivalents or *vice versa*.

$\dot{M}_{O_2}$ was calculated in $\mu mol\,kg^{-1}\,min^{-1}$ as:

$$\dot{M}_{O_2} = \frac{(P_{O_2,inc} - P_{O_2,exc})\beta_{O_2}F}{W}, \tag{2}$$

where β_{O_2} is the O_2 capacitance in fresh water at 21 °C (in $\mu mol\,l^{-1}\,kPa^{-1}$, taken from Dejours, 1981) and F is flow rate in $l\,min^{-1}$.

Similarly $\dot{M}_{CO_2}$ was calculated in $\mu mol\,kg^{-1}\,min^{-1}$ as:

$$\dot{M}_{CO_2} = \frac{(C_{CO_2,exc} - C_{CO_2,inc})F}{W}, \quad (3)$$

where C_{CO_2} is measured in $\mu mol\,l^{-1}$.

Finally the respiratory exchange ratio, R, was calculated as:

$$R = \dot{M}_{CO_2}/\dot{M}_{O_2}. \quad (4)$$

Statistical treatment

Data are expressed throughout as mean±S.E.M. (number of observations). Flux measurements were compared with zero by means of a modified *t*-test enabling a data set to be compared with a single point (Bailey, 1981). Statistical differences among treatment means were determined by one-way analysis of variance (dependent variable×time). When a significant F ratio was obtained, multiple comparisons were performed using Fisher's least significant difference (Ott, 1988). Statistical significance was accepted at $P<0.05$.

Results

Electrolyte fluxes and gas exchange rates are both typically expressed per unit wet mass. Comparing values during the moulting cycle presents a unique problem since mass does not remain constant in individuals. Water loading at ecdysis results in increased mass which does not necessarily reflect an increase in metabolizing tissue. To give some idea of the potential magnitude of this problem, female blue crabs (*Callinectes sapidus*) undergo a 47 % mass increase as they enter their terminal moult (Lewis and Haefner, 1976). Solutions commonly employed to circumvent this problem are to report individual whole-animal rates (Scudamore, 1947) or to correct for dry mass (Lewis and Haefner, 1976; Mangum *et al.* 1985). Whole-animal dry mass is not a good index to use since premoults will have a greater amount of inert material, some of which is shed at the moult. For the size of crayfish used in the present study, the mean increase in mass after ecdysis was only 8.4 %, which is within the S.E.M. for the range of mass for which data are reported. Therefore, we feel justified in expressing mass-specific data using the mass at the time measurements were obtained.

Electrolyte fluxes during the moulting cycle

The small net efflux of Ca^{2+} in intermoult crayfish (Fig. 1) was insignificantly different from zero ($P>0.05$), suggesting that they were in Ca^{2+} balance. This continued up until around 4 days premoult when a significant ($P=0.003$) net Ca^{2+} efflux of around $-800\,\mu mol\,kg^{-1}\,h^{-1}$ was measured. Following ecdysis, this changed vector to a substantial ($P<0.001$) net influx initially approaching

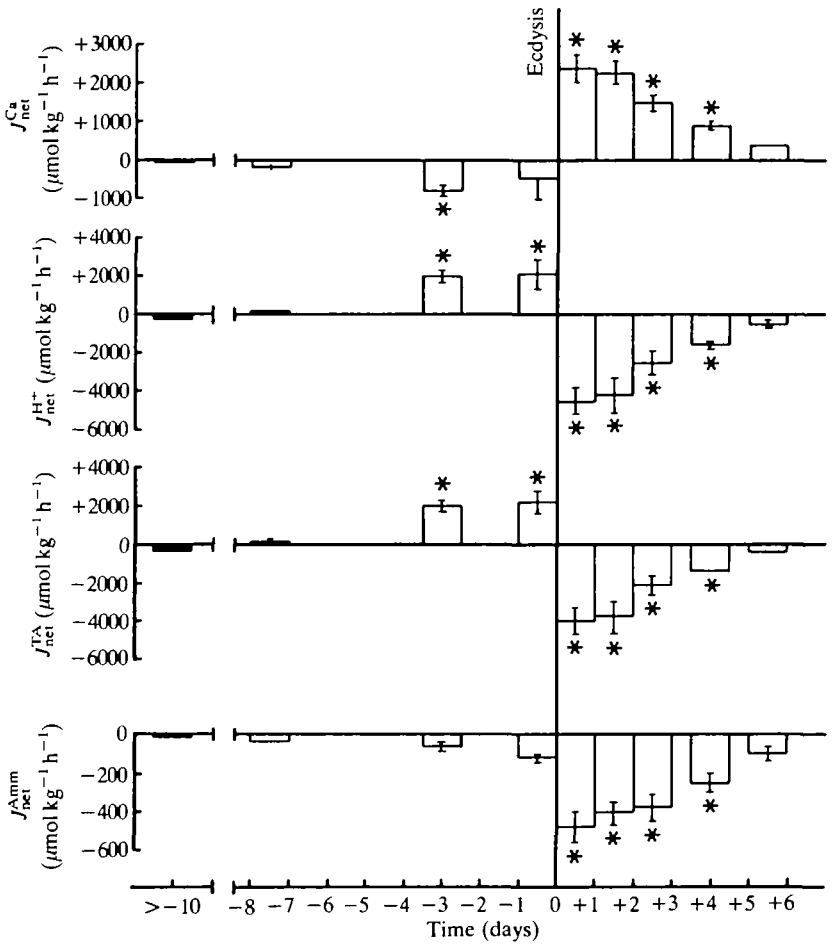

Fig. 1. Whole-animal net fluxes of Ca^{2+}, apparent H^+, titratable acidity (TA) and ammonia (Amm) with time throughout the moulting cycle in *Procambarus clarkii* (intermoult and premoult mean mass 1.66±0.18 g; postmoult mean mass 1.74±0.20 g) at 21°C. Ecdysis is indicated by the vertical line (t=0 day). Values are expressed as mean±S.E.M. (N=12 in premoult and 8 in postmoult). Wherever S.E.M. is not visible, it falls within the thickness of the line. By convention, positive fluxes indicate uptake by the crayfish and *vice versa*. Asterisks denote significance (P<0.05) compared with previous intermoult (more than 10 days prior to ecdysis, filled bars, extreme left).

+2500 μmol kg^{-1} h^{-1}. In most crayfish, uptake commenced after ecdysis; however, in some it was measured *prior* to shedding, explaining the large error bar on day t=−1. As postmoult progressed, the influx rate decreased. From day 3 onwards, influx rates had dropped significantly (P<0.001) below day 1 postmoult rates; however, they remained significantly (P<0.001) above intermoult rates for a further 2–3 days.

Intermoult crayfish exhibited a significant (P<0.001) net apparent H^+ output (or base uptake) of around −300 μmol kg^{-1} h^{-1} (Fig. 1), consisting primarily of

titratable acidic equivalents with only 3 % attributable to ammonia efflux. Around 3–4 days before ecdysis there was a significant ($P<0.001$) net H^+ influx (or base efflux) approaching $+2000\,\mu mol\,kg^{-1}\,h^{-1}$, which persisted in all crayfish until shedding occurred. The flux then promptly changed vector to a significant ($P<0.001$) H^+ efflux at initial rates exceeding $-4000\,\mu mol\,kg^{-1}\,h^{-1}$. These rates were significantly reduced by day 3 ($P<0.003$) although they continued to remain above intermoult levels for a further 2 days ($P<0.044$). By the end of day 6, intermoult fluxes had been re-established. Identical trends were observed for titratable acidity fluxes. Ammonia excretion was significant ($P<0.05$) in intermoult crayfish (Fig. 1) and did not change significantly during the premoult stages ($P>0.05$). However, during immediate postmoult there was a significant increase from around -120 to $-500\,\mu mol\,kg^{-1}\,h^{-1}$. After 2 days postmoult, the rates had fallen significantly ($P<0.02$) but remained above intermoult values for an additional 2 days ($P<0.001$). Six days after the moult, control (intermoult) levels of ammonia excretion had been restored ($P=0.189$).

The small net influxes of Na^+ and Cl^- in intermoult crayfish (Fig. 2) were both insignificantly different from zero ($P>0.05$), again suggesting ion balance. This persisted throughout the premoult phase, although there was less uniformity in the flux rates, especially in the day immediately preceding ecdysis. Individuals that had begun Ca^{2+} uptake prior to ecdysis also displayed net influx of both Na^+ and Cl^- (around $+500\,\mu mol\,kg^{-1}\,h^{-1}$), whereas those crayfish that continued to excrete Ca^{2+} immediately prior to shedding also exhibited net efflux of Na^+ and Cl^-. Immediately following ecdysis there was a significant net influx of both ions ($P<0.001$), and the influx remained elevated above control for an additional 2 days. While Na^+ influx remained at uniformly high levels ($P>0.21$), Cl^- influx decreased significantly on the second day ($P<0.01$), remaining at that level for the third day ($P=0.46$). In absolute terms, postmoult Cl^- influx rates were virtually double those of Na^+ ($+833$ and $+434\,\mu mol\,kg^{-1}\,h^{-1}$, respectively). After 3 days postmoult, Na^+ and Cl^- balance was re-established.

Intermoult crayfish were similarly in K^+ balance (Fig. 2) until 3–4 days preceding ecdysis, when a significant ($P<0.001$) efflux at rates of around $-70\,\mu mol\,kg^{-1}\,h^{-1}$ was measured until ecdysis. Immediately after ecdysis, there was a significant net uptake ($+14\,\mu mol\,kg^{-1}\,h^{-1}$) for 1 day ($P<0.04$). Thereafter, K^+ balance was restored.

Intermoult crayfish excreted significant ($P<0.01$) amounts of Mg^{2+} ($-21.4\pm5.9\,\mu mol\,kg^{-1}\,h^{-1}$) and sulphate ($-75.4\pm15.6\,\mu mol\,kg^{-1}\,h^{-1}$). Multiple comparisons failed to reveal any significant changes in either throughout the moulting cycle. Phosphate excretion (Fig. 3) was negligible ($P>0.10$) in inter- and premoult crayfish. During postmoult, there was a significant ($P<0.005$) phosphate excretion for the first two days.

Respiratory gas exchange during the moulting cycle

Intermoult crayfish had a mean $\dot{M}_{O_2}$ of $46\,\mu mol\,kg^{-1}\,min^{-1}$ and a corresponding $\dot{M}_{CO_2}$ of $57\,\mu mol\,kg^{-1}\,min^{-1}$, producing an R of 1.27 (Fig. 4). Analysis of variance

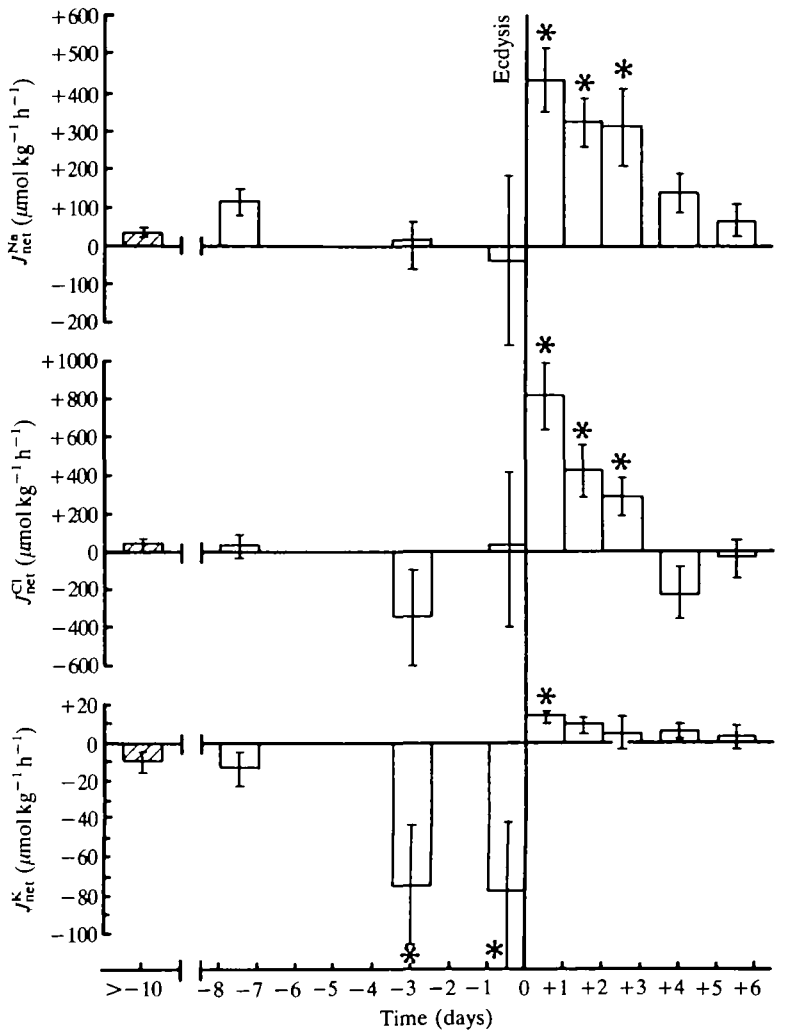

Fig. 2. Whole-animal net fluxes of Na^+, Cl^- and K^+ throughout the moulting cycle of *Procambarus clarkii* (mean mass 1.66±0.18 g; N=12). For additional details consult legend to Fig. 1. Previous intermoult data are shown as cross-hatched bars.

revealed significant changes in all three during the moulting cycle. $\dot{M}_{O_2}$ was unchanged until 2 days prior to ecdysis, when it increased to around 80 μmol kg^{-1} min^{-1}, remaining elevated (P<0.008) throughout the shedding process and into the first day postmoult, when it returned to intermoult levels. After 8 days postmoult, the values were significantly (P<0.02) below those of the previous intermoult.

$\dot{M}_{CO_2}$ similarly exhibited a doubling (P<0.001) of intermoult values in the immediate premoult period. During the entire postmoult period, $\dot{M}_{CO_2}$ was significantly (P<0.034) depressed below intermoult values. The combined effect

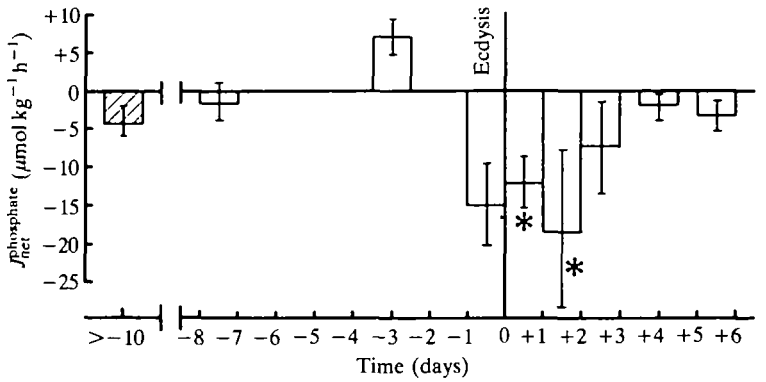

Fig. 3. Whole-animal net flux of phosphate throughout the moulting cycle of *Procambarus clarkii* (mean mass 1.66±0.18 g; N=12). For additional details consult legend to Fig. 1. Previous intermoult data are shown as cross-hatched bars.

of these changes was that R was significantly (P<0.011) lowered during the first 4 days of postmoult, although it had recovered by 8 days.

Discussion

This study has shown that the net fluxes of several major body electrolytes vary as a function of moulting stage in the freshwater crayfish. As crayfish approached premoult they switched from Ca^{2+} balance to net efflux (Fig. 1) at identical rates to those reported by Greenaway (1974*b*) in the crayfish *Austropotamobius pallipes*. In that study Greenaway calculated that 83 % of total body Ca^{2+} was lost at ecdysis; of this 56 % was in the exuviae and 27 % in soluble form. Thus Ca^{2+} storage in haemolymph and gastroliths (Travis, 1960, 1963) would appear to be relatively unimportant (17 % of total Ca^{2+}) compared with postmoult uptake from external sources.

In previous whole-animal studies (crayfish, Greenaway, 1974*c*; blue crab, Cameron, 1985, 1989) Ca^{2+} influx prior to shedding was never reported. However, Henry and Kormanik (1985) did report premoult Ca^{2+} uptake in isolated crab cuticle and Porcella *et al.* (1969) have reported that this occurred in *Daphnia*. Postmoult Ca^{2+} uptake rates were of the same order of magnitude as reported for crayfish in Greenaway (1975*c*). However, calcification was accomplished more rapidly in the present study (5 as opposed to 10 days) since the crayfish were smaller (2 as opposed to 10 g) and were maintained at a higher ambient temperature (21 compared to 10°C). Based on the existing chemical gradient, Ca^{2+} influx into the crayfish is necessarily by active uptake (Greenaway, 1985) and is elicited literally within minutes of shedding (Greenaway, 1974*c*). The uptake mechanism has not been identified but probably involves a Ca^{2+}-ATPase, if it is analogous with Ca^{2+} uptake across freshwater fish gills (Fenwick, 1978, modelled by Flik *et al.* 1985). By comparison, postmoult influx into the marine crab

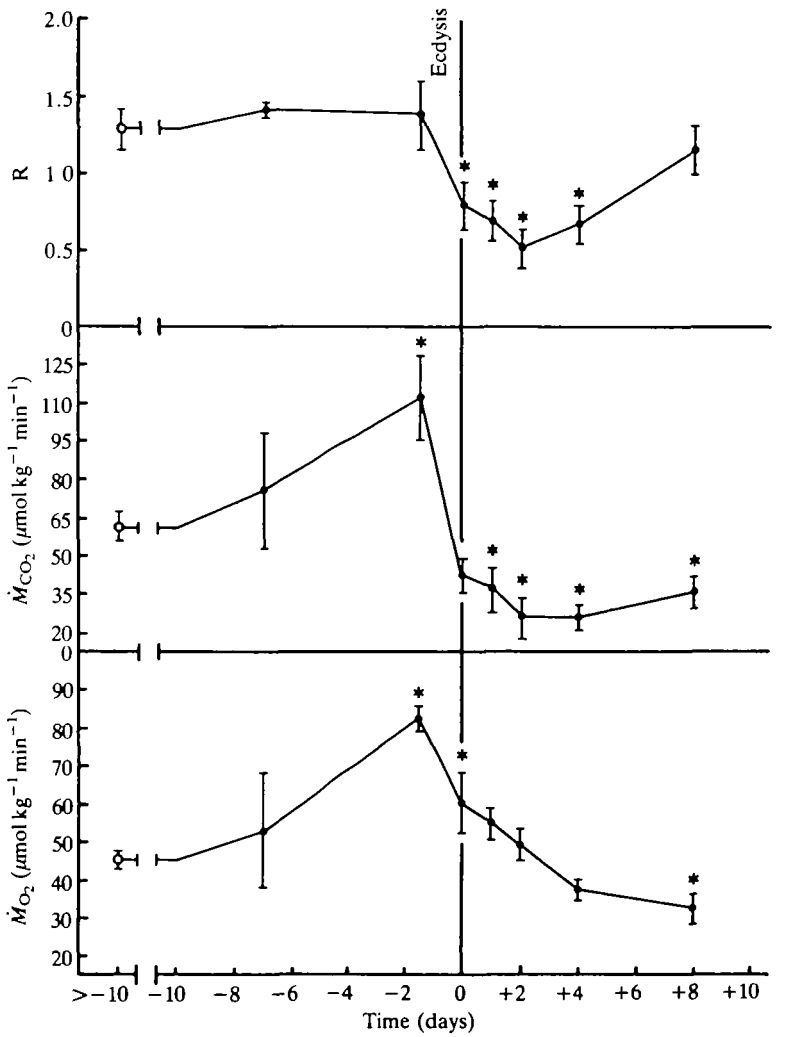

Fig. 4. Oxygen uptake rate ($\dot{M}_{O_2}$), carbon dioxide excretion rate ($\dot{M}_{CO_2}$) and apparent gas exchange ratio (R) with time throughout the moulting cycle in *Procambarus clarkii* (mean mass 1.71±0.14 g) at 21°C. Ecdysis is indicated by the vertical line. Values are expressed as mean±S.E.M. (N=12). Asterisks denote significance compared with previous intermoult (more than 10 days prior to ecdysis, open symbols).

Callinectes (≃200 g wet mass) was of the order of +8000 μmol kg^{-1} h^{-1} (Cameron, 1985) and did not commence until 12–24 h after ecdysis. Cameron (1989) has recently shown that this occurs by passive diffusion not active uptake; so the two processes do not appear to be analogous.

The apparent H^+ fluxes (Fig. 1) mirrored the net Ca^{2+} fluxes, suggesting that the transport mechanisms are linked. A small intermoult efflux of H^+ (alternatively HCO_3^- uptake) became a sizeable H^+ uptake (or HCO_3^- output) immediately prior to ecdysis. Resorption of cuticular $CaCO_3$ from the old skeleton

prior to ecdysis should produce Ca^{2+} and basic equivalents in the ratio of 1:2 (Cameron, 1985), which is the observed ratio. While it is tempting to conclude that the acid–base flux is a base efflux (probably HCO_3^-), the measurement technique does not differentiate between acid influx and base efflux or identify the ion. In the day immediately preceding ecdysis, the Ca^{2+} and acidic equivalent fluxes were not strongly correlated. All crayfish exhibited an influx of acidic equivalents even though Ca^{2+} uptake had commenced in certain individuals. During postmoult the observed fluxes were in the expected direction (i.e. Ca^{2+} uptake; H^+ output/HCO_3^- uptake) and similarly obeyed the stoichiometry of the calcification equation given in the Introduction. The fluxes also followed a similar time course, accomplishing calcification in approximately 5–6 days. The acid–base flux was attributable mainly to the titratable component; ammonia contributed little to the net flux. The postmoult increase in ammonia efflux may have been due to an increase in cuticular permeability or increased protein metabolism.

Intermoult crayfish exhibited a small net uptake of Na^+ and Cl^- (Fig. 2), as demonstrated in previous studies (Shaw, 1964; Ehrenfeld, 1974; Wheatly, 1989*b*); during most of premoult they remained in ion balance with respect to both ions. Net fluxes of Na^+ and Cl^- were variable on the day preceding ecdysis, yielding zero mean values. Uptake of Na^+ and Cl^- occurred in those individuals in which Ca^{2+} uptake had commenced (see above). Wherever Ca^{2+} efflux persisted, Na^+ and Cl^- net effluxes were also measured. This is strong evidence that part of whole-animal Ca^{2+} uptake is linked to uptake of Na^+ and Cl^-. This is in accordance with the view that Na^+ and Ca^{2+} fluxes across epithelial cells are related by a Na^+/Ca^{2+} exchanger (see review by Taylor and Windhager, 1979). Along these lines, Roer (1980) demonstrated that Ca^{2+} transport across an isolated crab cuticular hypodermis was Na^+-dependent.

Following ecdysis, there were large net influxes of both Na^+ and Cl^-, presumably to correct the haemodilution resulting from water uptake. Ion balance was restored within 3 days while calcification was still in progress. Although these fluxes were numerically only half the net Ca^{2+} influx rate, they are far larger than any recorded in response to acid–base disequilibria in the literature (Wood and Rogano, 1986; Wheatly, 1989*b*). Furthermore, assuming that unidirectional Na^+ efflux remained at intermoult values ($-300\,\mu mol\,kg^{-1}\,h^{-1}$ in the crayfish *Pacifastacus*; Wheatly, 1989*b*), unidirectional influx can be estimated as $+1000\,\mu mol\,kg^{-1}\,h^{-1}$, which is a substantial rate. Postmoult net Cl^- fluxes were higher than Na^+ fluxes, possibly reflecting greater diffusional permeability. The evidence from unidirectional flux studies on other crayfish species (*Orconectes*, Wood and Rogano, 1986; *Pacifastacus*, Wheatly, 1989*b*), indicates that the integument is more permeable to Cl^- than to Na^+. Again, assuming a control unidirectional Cl^- efflux of $-500\,\mu mol\,kg^{-1}\,h^{-1}$, postmoult Cl^- influx rate can be estimated as $+1300\,\mu mol\,kg^{-1}\,h^{-1}$. In the likely event that diffusional efflux increases at ecdysis, unidirectional influxes of both ions would be even greater than predicted.

The switch from K^+ balance to a sizeable net efflux (Fig. 2) in the 3 days prior to

ecdysis deserves explanation. The extracellular fluid compartment in the carapace selectively concentrates electrolytes, including K^+ (M. G. Wheatly, unpublished observations). Little is known of the carapace fluid dynamics during the moult but it is likely that fluid is reabsorbed as the old skeleton is degraded. In addition, atrophy of various somatic muscle cells (Skinner, 1985) could contribute to high circulating K^+ levels which would result in increased diffusional loss. The initial postmoult uptake is insufficient to make up for the premoult loss, from which one must conclude that K^+ is obtained over the long term in the diet.

The significant postmoult phosphate excretion (Fig. 3) may have been attributable to enhanced diffusional permeability before the new exoskeleton had hardened. Alternatively it may have resulted from increased urinary flow. While this has never been measured in freshly moulted decapods, the increase in hydrostatic pressure at ecdysis should increase filtration pressure (Mykles, 1980; deFur *et al.* 1985) and thereby urine flow. Because of large variability between animals, similar trends could not be demonstrated for Mg^{2+} and sulphate, which are also prominent components of both haemolymph and urine (Wheatly and Toop, 1989).

Intermoult $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ were not significantly different (Fig. 4). The fact that R exceeded unity (1.27) may have arisen from aeration of the flux water which would have reduced ambient P_{CO_2}, thus facilitating CO_2 excretion. The only R value in the literature for which $\dot{M}_{CO_2}$ had been measured and not estimated was 0.6, which was calculated for the land crab *Cardisoma* by Wood and Randall (1981). They attributed the low value to retention of respiratory CO_2 for carapace formation. However, during exercise R did rise above 1 in *Cardisoma*, confirming that this can occur in decapods. Owing to the small size range of the crayfish used in this study, absolute values for $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ cannot be compared with the majority of existing data. The $\dot{M}_{O_2}$ values were of the same order of magnitude as values measured in similarly sized *Pacifastacus leniusculus* (Wheatly, 1989*a*).

The rate of O_2 consumption has been measured during the moulting cycle in the crayfish (Scudamore, 1947) as well as certain marine decapods (Lewis and Haefner, 1976; Penkoff and Thurberg, 1982; Mangum *et al.* 1985). Common to all the studies was a doubling of $\dot{M}_{O_2}$ around the time of ecdysis, although the precise timing of the peak rate varied. The present finding that $\dot{M}_{O_2}$ is highest immediately premoult (Fig. 4) agrees with certain studies (Scudamore, 1947; Penkoff and Thurberg, 1982). Some (Scudamore, 1947; Lewis and Haefner, 1976) have suggested that the rate drops during the actual shedding process, based on measurements made using closed respirometers. Some authors have reported elevated postmoult rates for up to 2 days (Scudamore, 1947; Penkoff and Thurberg, 1982; Mangum *et al.* 1985). In the present study the mass-specific $\dot{M}_{O_2}$ returned to control levels within a day. By 8 days postmoult, $\dot{M}_{O_2}$ had dropped significantly below the previous intermoult value as reported by Penkoff and Thurberg (1982); when converted to whole-animal rates there was no difference in successive intermoult rates, suggesting that this was an artefact of the increase in mass due to water loading.

$\dot{M}_{CO_2}$ dropped significantly below $\dot{M}_{O_2}$ for the first 4 days postmoult, possibly representing the net result of HCO_3^- uptake countering CO_2 elimination. Assuming that R remained at 1 for cellular respiration, then an $\dot{M}_{CO_2}$ deficit of 1178 μmol kg^{-1} h^{-1} (2356 μequiv kg^{-1} h^{-1}) can be calculated. This is of the same order of magnitude as the uptake of basic equivalents illustrated in Fig. 1, given that measurements were on separate experimental series. The 'apparent' respiratory exchange ratio meanwhile dropped (Fig. 4) while calcification was underway. In the 24 h preceding ecdysis, the $\dot{M}_{CO_2}$ excess was 1800 μmol kg^{-1} h^{-1}, which is similar to the measured output of basic equivalents (Fig. 1). Cameron and Wood (1985) measured a net CO_2 uptake from sea water in postmoult blue crabs, concluding that the CO_2 source for calcification was at least partly external. Subsequent measurements of P_{CO_2} failed to reveal that the carapace was a sink for metabolic CO_2 (Cameron, 1985). This suggests that the external water provided the majority of the CO_2 in the form of HCO_3^-. Uptake of HCO_3^-, like Ca^{2+} uptake, proceeds at a relatively reduced rate in crayfish compared with the blue crab; this may reflect the lowered external HCO_3^- concentration or some direct or indirect limitation to the uptake mechanism. Postmoult ion uptake mechanisms are examined in greater detail in a separate paper (M. G. Wheatly and A. T. Gannon, in preparation).

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